OA OSTEOARTHRITIS

A1

CALCITONIN DIRECTLY ATTENUATES COLLAGEN TYPE II DEGRADATION BY INHIBITION OF MMP EXPRESSION AND ACTIVITY IN ARTICULAR CHONDROCYTES

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Purpose: Calcitonin was recently shown to counter the progression of cartilage degradation in experimental models of osteoarthritis. The beneficial effect was believed mediated indirectly by inhibition of subchondral bone resorption. We investigated whether calcitonin has direct effect on articular cartilage chondrocytes.
Methods: The localisation and expression of the calcitonin receptor in articular chondrocytes was investigated by immunhistochemistry and RT-PCR. Potential direct effects was tested in the articular cartilage explants model, where cartilage degradation was induced by cytokine stimulation of TNF-α [20ng/ml] + oncostatin M (OSM) [10ng/ml], and cultured with salmon or human calcitonin simultaneously [0.0001-1mM]. The changes in cartilage degradation were investigated in the conditioned medium by quantification of C-terminal telopeptides of collagen type II (CTX-II) and sulphated glycosaminoglycans release (sGAG) was tested by the Wielisa®-Kit. The effect of calcitonin was investigated *in vivo* using rats. Rats were administered with an oral dose of calcitonin (2 mg/kg) bound to the carrier 5-CNAC (150 mg/kg). Collagen type II degradation was quantified in serum by measuring the released CTX-II and the degradation of bone by CTX-I, a marker of bone resorption, measuring fragments of collagen type I.
Results: The calcitonin receptor was identified on articular chrondrocytes, both mRNA and protein forms. Culturing articular cartilage explants in the presence of TNF-a and OSM resulted in a marked, 100-fold increase in CTX-II release compared to vehicle-treated controls ($p<0.001$). Addition of human or salmon calcitonin to the explants culture [0.0001-1mM] in the concomitant presence of TNF-α and OSM resulted in a significant and dose dependent inhibition of CTX-II release ($p<0.01$). At 100 nm and 1 mM of calcitonin treatment in the presence of OSM and TNF-α, calcitonin very nearly abrogated collagen type II release, at day 13,16 and 19 of culture. In the absence of OSM and TNF-α, calcitonin significantly inhibited GAG release into the conditioned medium. Proteolytic activity was investigated by zymography. TNF-α and OSM resulted in a strong up regulation of MMP-9 activity and expression. This increase in MMP activity was strongly attenuated by calcitonin at 100 nM and 1 mM, and the positive control GM6001 [25 mM], a the general MMP inhibitor, Under *in vivo* conditions, 4-day oral treatment with calcitonin induced a 95% decrease in serum CTX-II levels, i.e. chondrocyte-mediated cartilage degradation. The parallel effects of calcitonin treatment on bone resorption was indicated by a 50% decrease in urinary CTX-I excretion.
Conclusions: These results suggest that calcitonin 1) acts directly on articular chondrocytes; 2) inhibits MMP expression and the related collagen type II degradation; and 3) carries potentials for becoming a useful therapeutical option for patients with degenerative joint diseases.

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GADD45β IS NOVEL MEDIATOR OF MMP-13 GENE EXPRESSION DURING CHONDROCYTE HYPERTROPHY

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Aim of Study: To define the role of growth arrest and DNA damage-inducible 45β (GADD45β) in the regulation of chondrocyte differentiation in the embryonic growth plate.
Methods: Immortalized human chondrocytes (C-28/I2) were treated with BMP-2 at 100 ng/ml for 1 h and total RNA was analyzed on the Affymetrix Gene Chip Human Genome U133A microarray and the highly expressed genes were confirmed in C28/I2 cells and primary human chondrocytes by real-time PCR. ATDC5 cells and pellet cultures of murine rib growth plate chondrocytes were grown under defined conditions as models of chondrogenesis. Lentiviral siRNA delivery was performed to knockdown GADD45β and the pcDNA3-Gadd45β-flag expression vector was used to overexpress GADD45β. GADD45β and MMP-13 promoter activities were assessed using luciferase reporter constructs. Mouse embryonic growth plates were examined by immunohistochemistry and in situ hybridization.
Results: We identified GADD45β as a prominent early response gene induced by BMP-2 through a Smad1/Runx2-dependent pathway. Since this pathway is involved in skeletal development, we examined embryonic growth plates and observed expression of GADD45β mRNA coincident with Runx2 protein in pre-hypertrophic chondrocytes, whereas GADD45β protein was localized in the nucleus in late stage hypertrophic chondrocytes where Mmp13 mRNA was expressed. In Gadd45β knockout mouse embryos, defective mineralization and decreased endochondral growth accompanied deficient Mmp13 and Col10a1 gene expression in the hypertrophic zone. Transduction of siRNA-GADD45β in epiphyseal chondrocytes in vitro blocked terminal differentiation and the associated expression of Mmp13 and Col10a1 mRNA. Finally, GADD45β stimulated MMP-13 promoter activity in chondrocytes through c-Jun N-terminal kinase (JNK)-mediated phosphorylation of JunD partnered with Fra2 in synergy with Runx2.
Discussion: Our results demonstrate a previously undiscovered role for GADD45β, which has been implicated in the stress response and cell survival during terminal differentiation of other cell types, as a critical mediator during endochondral ossification. The intracellular accumulation of GADD45β protein in intact hypertrophic chondrocytes in the mouse embryonic growth plate and the effects of GADD45β deficiency in vivo and in vitro indicate that GADD45β is required for MMP-13 expression during the later stages of chondrocyte hypertrophy. Our findings on Runx2 synergy provide a new concept regarding a temporal and spatial link between BMP-2-induced GADD45β and the induction of MMP-13 gene transcription via the JNK/JunD pathway at terminal stages. Furthermore, as we reported previously, GADD45β is expressed in normal cartilage, increased in early stage osteoarthritis (OA), and decreased in late stage OA, particularly in the deep, calcified zone, where it may serve as an anti-apoptotic signal in cells with features of hypertrophic chondrocytes, suggesting a role in tidemark advancement.